

# Supplementary Information

## **On-tissue amidation of sialic acid with aniline for sensitive imaging of sialylated N-glycans from FFPE tissue sections via MALDI mass spectrometry**

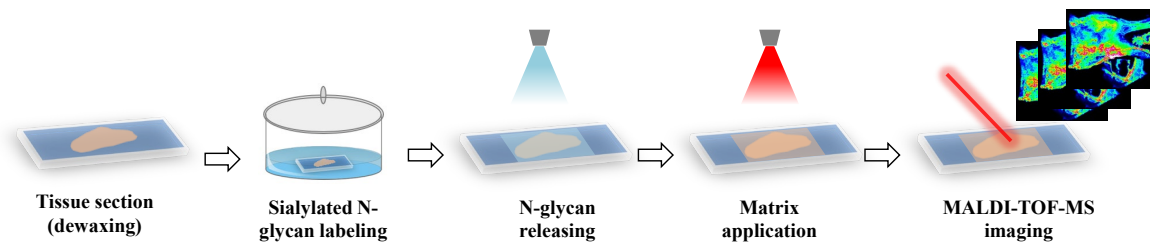
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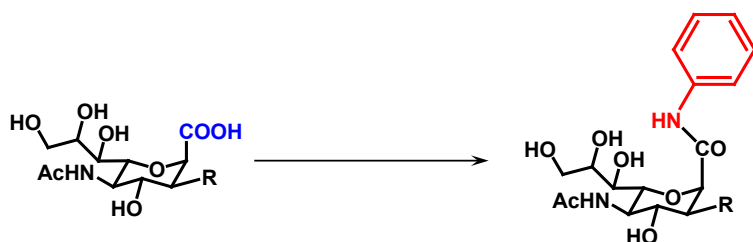
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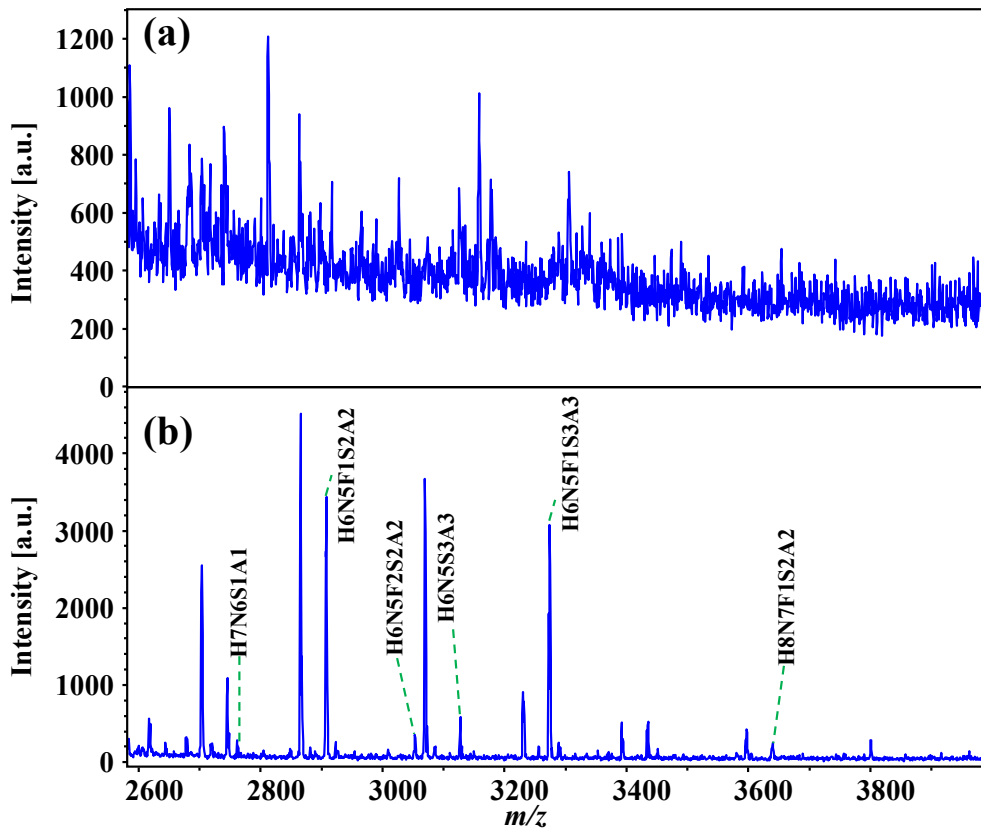
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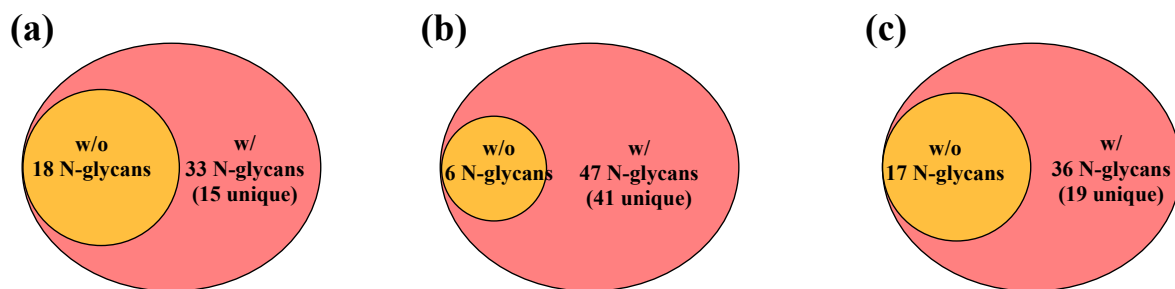
**Figure S1 Schematic diagram of the on-tissue labeling of sialylated N-glycans using aniline for MALDI-MS imaging.**



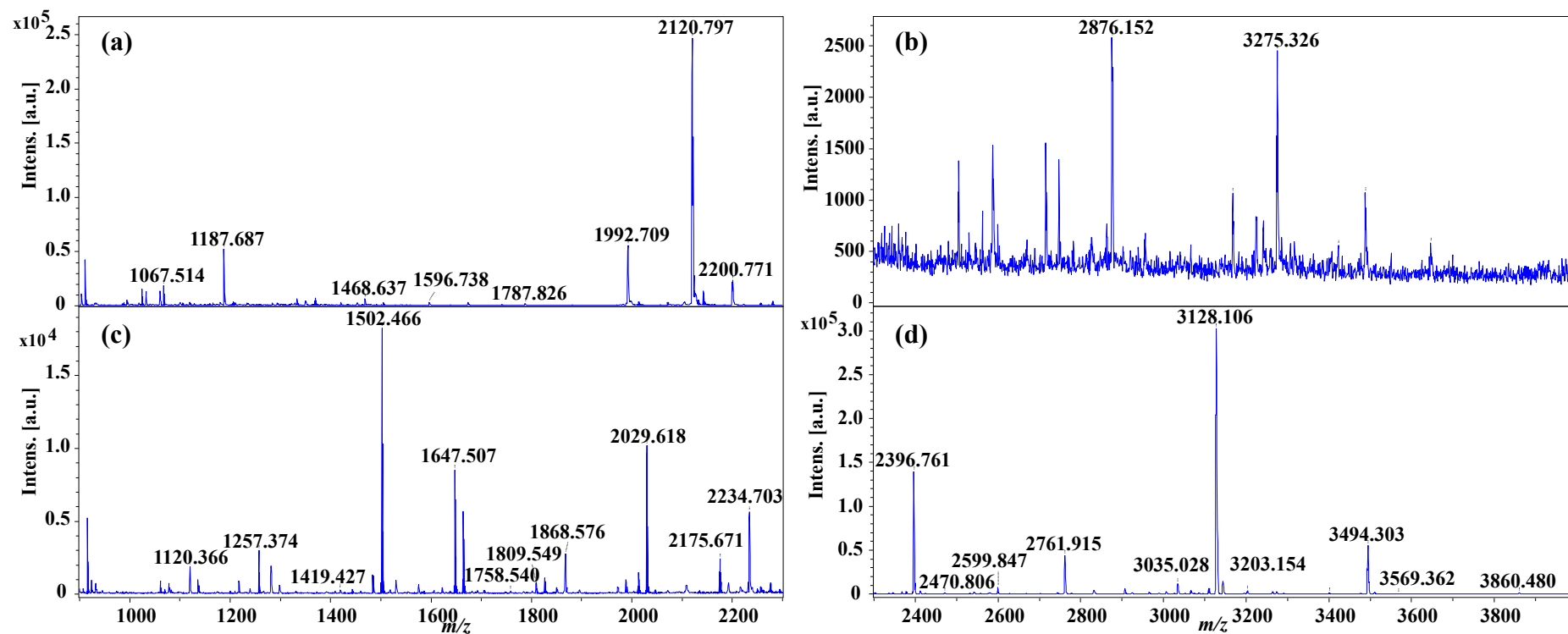
**Scheme S1. The chemical reaction of labeling the sialic acid residue of sialylated N-glycan with aniline.**



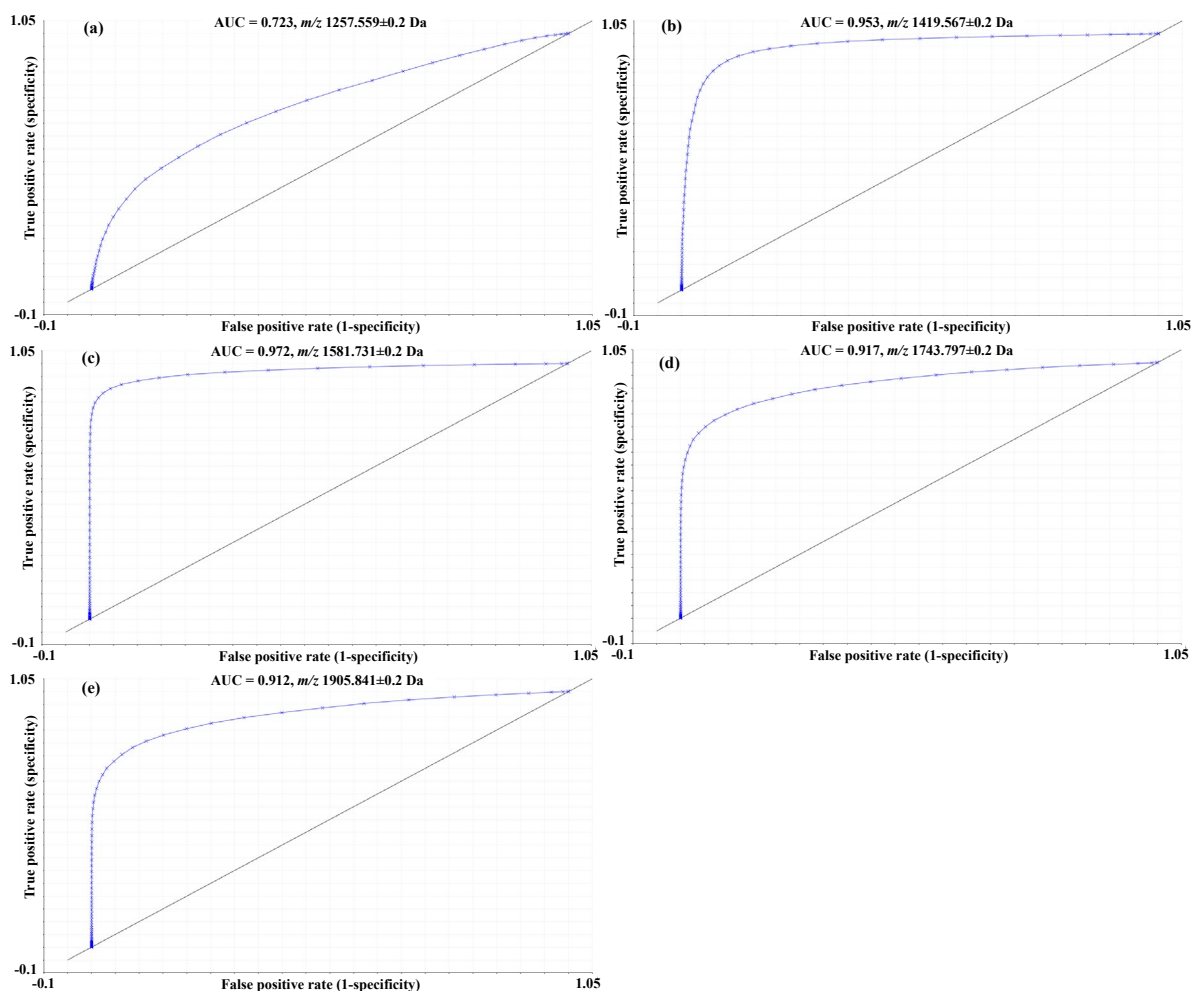
**Figure S2. Zoomed-in mass spectra of BTG glycoprotein over a mass range of 2600-4000 Da:** (a) sample without derivatization, (b) sample with aniline amidation derivatization. N-glycan units include Hexose (H), HexNAc (N), Fucose (F), Sialic acid (S), tagged aniline (A).



**Figure S3. Venn diagrams presenting the numbers of N-glycans detected in the analysis of glycoproteins and tissue section:** (a) analysis of bovine thyroglobulin protein without (w/o) and with (w/) aniline amidation derivatization, (b) analysis of fetuin protein without (w/o) and with (w/) aniline amidation derivatization, (c) N-glycans detected from one specific region of the human laryngeal tissue without (w/o) and with (w/) chemical derivatization.



**Figure S4. Zoomed-in mass spectra of fetuin glycoprotein: (a) over a mass range of 900-2300 Da for sample without derivatization, (b) over a mass range of 2300-4000 Da for sample without derivatization, (c) over a mass range of 900-2300 Da for sample with aniline amidation derivatization, (d) over a mass range of 2300-4000 Da for sample with aniline amidation derivatization.**



**Figure S5. ROC curves of cancer region plotted against normal region of the laryngeal tissue sample at ion intensities of N-glycans of (a) Hex<sub>5</sub>HexNAc<sub>2</sub> at  $m/z$  1257.559, (b) Hex<sub>6</sub>HexNAc<sub>2</sub> at  $m/z$  1419.567, (c) Hex<sub>7</sub>HexNAc<sub>2</sub> at  $m/z$  1581.731, (d) Hex<sub>8</sub>HexNAc<sub>2</sub> at  $m/z$  1743.797, (e) Hex<sub>9</sub>HexNAc<sub>2</sub> at  $m/z$  1905.841.**

**Table S1. N-glycans detected from BTG glycoprotein using MALDI-TOF-MS without aniline labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	1257.322	145.7	Hex5HexNAc2	1257.423
2	1282.363	13.3	Hex3HexNAc3dHex1	1282.455
3	1419.361	41.6	Hex6HexNAc2	1419.476
4	1444.394	24.9	Hex4HexNAc3dHex1	1444.508
5	1581.399	70.8	Hex7HexNAc2	1581.529
6	1647.452	10.7	Hex4HexNAc4dHex1	1647.587
7	1663.454	6.4	Hex5HexNAc4	1663.582
8	1743.442	103.0	Hex8HexNAc2	1743.582
9	1751.700	18.9	Hex5HexNAc3NeuAc1	1751.598
10	1809.506	69.2	Hex5HexNAc4dHex1	1809.640
11	1825.474	6.5	Hex6HexNAc4	1825.635
12	1905.479	202.5	Hex9HexNAc2	1905.634
13	1971.547	71.8	Hex6HexNAc4dHex1	1971.693
14	2012.556	3.5	Hex5HexNAc5dHex1	2012.719
15	2262.577	7.3	Hex6HexNAc4NeuAc1dHex1	2262.788
16	2336.655	8.8	Hex7HexNAc5dHex1	2336.825
17	2450.151	17.3	Hex5HexNAc5NeuAc1dHex2	2449.873
18	2500.729	7.5	Hex3HexNAc9dHex1	2500.931

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)

**Table S2. N-glycans detected from BTG glycoprotein using MALDI-TOF-MS with aniline labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	1257.331	27.0	Hex5HexNAc2	1257.423
2	1282.361	28.1	Hex3HexNAc3dHex1	1282.455
3	1419.374	10.7	Hex6HexNAc2	1419.476
4	1444.398	28.1	Hex4HexNAc3dHex1	1444.508
5	1581.411	20.5	Hex7HexNAc2	1581.529

6	1647.452	17.2	Hex4HexNAc4dHex1	1647.587
7	1663.452	3.9	Hex5HexNAc4	1663.582
8	1743.446	53.6	Hex8HexNAc2	1743.582
9	1809.495	15.9	Hex5HexNAc4dHex1	1809.640
10	1825.506	3.5	Hex6HexNAc4	1825.635
11	1826.495	12.7	Hex5HexNAc3NeuAc1	1826.645
12	1867.522	6.2	Hex4HexNAc4NeuAc1	1867.672
13	1905.491	175.3	Hex9HexNAc2	1905.634
14	1971.532	35.6	Hex6HexNAc4dHex1	1971.693
15	1988.536	7.6	Hex6HexNAc3NeuAc1	1988.698
16	2012.551	3.6	Hex5HexNAc5dHex1	2012.719
17	2030.565	8.1	Hex4HexNAc3NeuAc2	2030.735
18	2134.585	7.9	Hex6HexNAc3NeuAc1dHex1	2134.756
19	2174.595	18.3	Hex6HexNAc5dHex1	2174.772
20	2336.655	15.1	Hex7HexNAc5dHex1	2336.825
21	2337.669	767.8	Hex6HexNAc4NeuAc1dHex1	2337.835
22	2393.694	4.0	Hex7HexNAc6	2393.846
23	2394.674	6.2	Hex6HexNAc5NeuAc1	2394.857
24	2500.733	9.5	Hex3HexNAc9dHex1	2500.931
25	2524.751	26.9	Hex5HexNAc5NeuAc1dHex2	2524.920
26	2541.764	1249.6	Hex5HexNAc4NeuAc2dHex1	2541.925
27	2556.740	6.4	Hex7HexNAc5NeuAc1	2556.910
28	2760.861	7.4	Hex7HexNAc6NeuAc1	2759.989
29	2906.919	77.1	Hex6HexNAc5NeuAc2dHex1	2907.057
30	3053.979	9.1	Hex6HexNAc5NeuAc2dHex2	3053.115
31	3127.021	10.4	Hex6HexNAc5NeuAc3	3127.142
32	3273.101	61.6	Hex6HexNAc5NeuAc3dHex1	3273.200
33	3638.289	6.2	Hex8HexNAc7NeuAc2dHex1	3637.322

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)

**Table S3. N-glycans detected from fetuin glycoprotein using MALDI-TOF-MS without aniline labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	933.399	6.5	Hex3HexNAc2	933.318



2	1079.511	3.4	Hex3HexNAc2dHex1	1079.375
3	1420.616	12.0	Hex6HexNAc2	1419.476
4	2012.601	6.2	Hex5HexNAc5dHex1	2012.719
5	2141.785	56.3	Hex4HexNAc5NeuAc1dHex1	2141.762
6	2256.809	12.8	Hex4HexNAc7dHex1	2256.825

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetylneuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)

**Table S4. N-glycans detected from fetuin glycoprotein using MALDI-TOF-MS with aniline labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	933.294	8.2	Hex3HexNAc2	933.318
2	1079.330	10.3	Hex3HexNAc2dHex1	1079.375
3	1136.358	23.0	Hex3HexNAc3	1136.397
4	1257.374	71.0	Hex5HexNAc2	1257.423
5	1282.401	33.6	Hex3HexNAc3dHex1	1282.455
6	1298.395	14.9	Hex4HexNAc3	1298.450
7	1419.427	7.1	Hex6HexNAc2	1419.476
8	1444.445	5.9	Hex4HexNAc3dHex1	1444.508
9	1485.466	27.8	Hex3HexNAc4dHex1	1485.534
10	1502.466	412.4	Hex3HexNAc3NeuAc1	1502.540
11	1622.476	9.7	Hex6HexNAc3	1622.555
12	1648.509	139.2	Hex3HexNAc3NeuAc1dHex1	1648.598
13	1664.503	121.8	Hex4HexNAc3NeuAc1	1664.592
14	1809.548	14.5	Hex5HexNAc4dHex1	1809.640
15	1810.549	15.5	Hex4HexNAc3NeuAc1dHex1	1810.650
16	1826.549	21.8	Hex5HexNAc3NeuAc1	1826.645
17	1850.563	9.3	Hex4HexNAc5dHex1	1850.666
18	1867.570	53.5	Hex4HexNAc4NeuAc1	1867.672
19	1972.595	7.5	Hex5HexNAc3NeuAc1dHex1	1972.703
20	1988.598	18.3	Hex6HexNAc3NeuAc1	1988.698
21	2012.622	9.5	Hex5HexNAc5dHex1	2012.719
22	2029.618	185.5	Hex5HexNAc4NeuAc1	2029.725
23	2175.671	40.3	Hex5HexNAc4NeuAc1dHex1	2175.783
24	2192.668	12.5	Hex5HexNAc3NeuAc2	2192.788

25	2216.681	7.6	Hex4HexNAc5NeuAc1dHex1	2216.809
26	2232.686	13.5	Hex5HexNAc5NeuAc1	2232.804
27	2256.680	7.8	Hex4HexNAc7dHex1	2256.825
28	2304.701	12.9	Hex5HexNAc5dHex3	2304.835
29	2336.728	8.6	Hex7HexNAc5dHex1	2336.825
30	2337.724	12.9	Hex6HexNAc4NeuAc1dHex1	2337.835
31	2395.759	1891.7	Hex5HexNAc4NeuAc2	2395.867
32	2541.821	36.4	Hex5HexNAc4NeuAc2dHex1	2541.925
33	2557.815	9.4	Hex6HexNAc4NeuAc2	2557.920
34	2598.843	65.8	Hex5HexNAc5NeuAc2	2598.947
35	2743.902	16.9	Hex6HexNAc6NeuAc1dHex1	2743.994
36	2759.899	16.6	Hex7HexNAc6NeuAc1	2759.989
37	2907.983	94.3	Hex5HexNAc4NeuAc3dHex1	2908.068
38	3053.044	12.6	Hex6HexNAc5NeuAc2dHex2	3053.115
39	3110.086	100.7	Hex6HexNAc6NeuAc2dHex1	3110.137
40	3127.102	3198.0	Hex6HexNAc5NeuAc3	3127.142
41	3264.138	43.3	Hex4HexNAc8NeuAc1dHex4	3264.221
42	3273.175	33.1	Hex6HexNAc5NeuAc3dHex1	3273.200
43	3289.177	7.7	Hex7HexNAc5NeuAc3	3289.195
44	3331.222	4.7	Hex5HexNAc5NeuAc4	3331.232
45	3476.285	14.0	Hex6HexNAc6NeuAc3dHex1	3476.280
46	3493.297	528.9	Hex6HexNAc5NeuAc4	3493.285
47	3858.487	7.3	Hex7HexNAc6NeuAc4	3858.417

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)

**Table S5. N-glycans detected from tissue sample using MALDI-TOF-MS without aniline labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	1079.103	13.8	Hex3HexNAc2dHex1	1079.375
2	1257.391	115.9	Hex5HexNAc2	1257.423
3	1419.446	375.7	Hex6HexNAc2	1419.476
4	1485.488	8.4	Hex3HexNAc4dHex1	1485.534
5	1581.487	83.5	Hex7HexNAc2	1581.529
6	1647.529	15.5	Hex4HexNAc4dHex1	1647.587

7	1663.532	7.4	Hex5HexNAc4	1663.582
8	1688.552	4.7	Hex3HexNAc5dHex1	1688.614
9	1704.543	4.3	Hex4HexNAc5	1704.609
10	1743.525	59.6	Hex8HexNAc2	1743.582
11	1809.569	62.5	Hex5HexNAc4dHex1	1809.640
12	1850.580	37.3	Hex4HexNAc5dHex1	1850.666
13	1905.675	12.5	Hex9HexNAc2	1905.634
14	1996.627	63.2	Hex4HexNAc5dHex2	1996.724
15	2012.792	5.1	Hex5HexNAc5dHex1	2012.719
16	2028.712	4.3	Hex6HexNAc5	2028.714
17	2141.609	3.1	Hex4HexNAc5NeuAc1dHex1	2141.762

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)

**Table S6. N-glycans detected from tissue sample using MALDI-TOF-MS with aniline**

**labeling**

No	Experimental mass	Signal-to-noise (SN)	Identified N-glycans <sup>a</sup>	Theoretical adduct mass <sup>b</sup>
1	1079.122	13.6	Hex3HexNAc2dHex1	1079.375
2	1257.423	37.8	Hex5HexNAc2	1257.423
3	1419.444	98.1	Hex6HexNAc2	1419.476
4	1444.488	4.1	Hex4HexNAc3dHex1	1444.508
5	1445.466	6.3	Hex3HexNAc2NeuAc1dHex1	1445.518
6	1485.484	6.5	Hex3HexNAc4dHex1	1485.534
7	1501.671	5.6	Hex4HexNAc4	1501.529
8	1581.482	26.1	Hex7HexNAc2	1581.529
9	1622.514	4.3	Hex6HexNAc3	1622.555
10	1647.534	15.6	Hex4HexNAc4dHex1	1647.587
11	1648.531	9.3	Hex3HexNAc3NeuAc1dHex1	1648.598
12	1663.524	6.0	Hex5HexNAc4	1663.582
13	1664.543	6.3	Hex4HexNAc3NeuAc1	1664.592
14	1688.563	5.1	Hex3HexNAc5dHex1	1688.614
15	1704.541	4.7	Hex4HexNAc5	1704.609
16	1743.514	16.3	Hex8HexNAc2	1743.582
17	1809.574	26.5	Hex5HexNAc4dHex1	1809.640
18	1850.586	14.5	Hex4HexNAc5dHex1	1850.666

19	1867.577	3.5	Hex4HexNAc4NeuAc1	1867.672
20	1905.569	3.8	Hex9HexNAc2	1905.634
21	1955.604	4.3	Hex5HexNAc4dHex2	1955.698
22	1996.644	19.5	Hex4HexNAc5dHex2	1996.724
23	2012.612	5.1	Hex5HexNAc5dHex1	2012.719
24	2013.622	7.2	Hex4HexNAc4NeuAc1dHex1	2013.730
25	2028.642	3.6	Hex6HexNAc5	2028.714
26	2029.635	5.2	Hex5HexNAc4NeuAc1	2029.725
27	2175.673	40.3	Hex5HexNAc4NeuAc1dHex1	2175.783
28	2216.684	13.5	Hex4HexNAc5NeuAc1dHex1	2216.809
29	2378.737	3.5	Hex5HexNAc5NeuAc1dHex1	2378.862
30	2395.728	9.7	Hex5HexNAc4NeuAc2	2395.867
31	2541.795	24.9	Hex5HexNAc4NeuAc2dHex1	2541.925
32	2581.802	4.0	Hex5HexNAc6NeuAc1dHex1	2581.941
33	2761.195	3.1	Hex6HexNAc5NeuAc2	2761.000
34	2907.933	25.4	Hex5HexNAc4NeuAc3dHex1	2908.068
35	3272.135	5.6	Hex7HexNAc6NeuAc2dHex1	3272.190
36	3273.128	11.4	Hex6HexNAc5NeuAc3dHex1	3273.200

<sup>a</sup> Hex, hexose (galactose, mannose); HexNAc, HexNAC, N-acetylhexosamine; NeuAc, N-acetyl neuraminic acid; dHex, fucose

<sup>b</sup> Theoretical adduct *m/z* refers to databases of GlycoWorkbench (<https://code.google.com/archive/p/glycoworkbench/>)